



NTP
National Toxicology Program

Development of a Short-term Cancer Bioassay Using Multiple p53 Haploinsufficient F1 Inbred Strains

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Background and Rationale

- Genomic instability and allelic loss (loss of heterozygosity or LOH) and loss of tumor suppressor genes are a hallmark of cancer
- TSG function may result from mutation and/or epigenetic suppression
- GAMM models show potential for predicting human disease
- Development of a rapid and predictive model for non-random loss of tumor suppressor gene function will
 - ✓ Identify a mode of action for hazard characterization,
 - ✓ reduce potential for false positives and false negative,
 - ✓ and aid extrapolation of risk to human populations.



Are genetically-altered mouse models more accurate?

Data set for prediction (99 test chemicals)

B6.129-*Trp53*^{tm1Brd}

FVB/N-Tg(*vHRas*)^{4Lep}

CB6F1-Tg(*HRAS*)²

[+]

- 47 IARC Group 1/2 NTP ROC -
Known/Probable Human Carcinogens

[-]

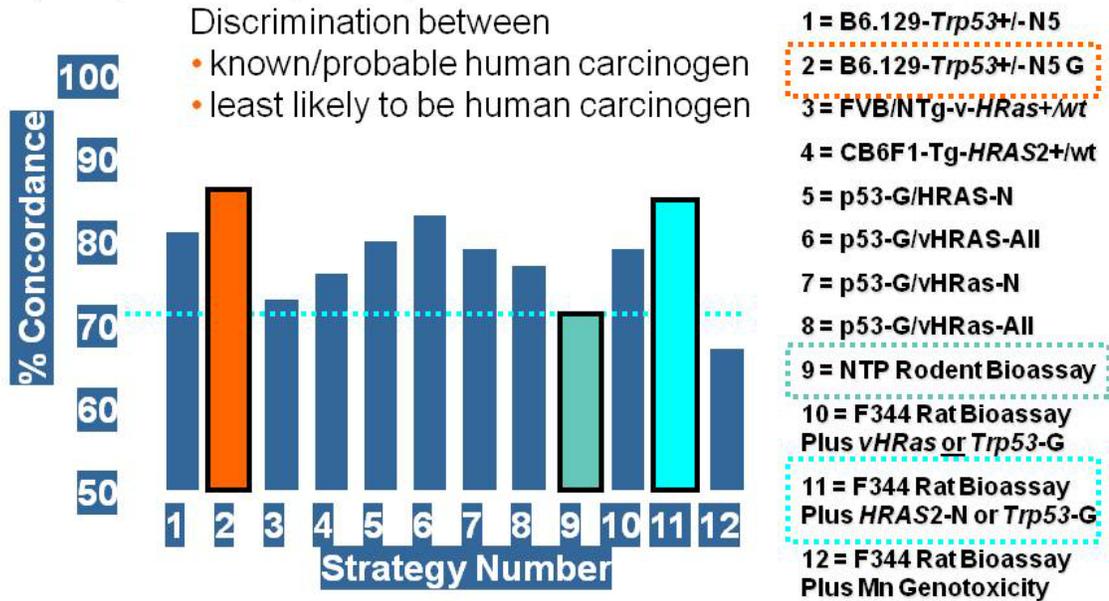
- 52 IARC Class 3/NTP ROC negative/unlisted
Least likely to be a human carcinogen

Pritchard, French, Davis & Haseman, *Env Health Persp* 111:444 (2003)



GAMM Cancer Bioassay Predictability

(26 wk, not 39 wk exposures)



Pritchard, French, Davis & Haseman, *Env Health Persp* 111:444 (2003)

NTP studies with GM Models for Toxicology & Carcinogenesis (39 wks treatment)

No.	Agent	Model	Dosage (Route)	Results
1	Aspartame	B6.129-Trp53tm1Brd	(feed)	Negative
		B6.129-Cdkn2atm1Rdp	(feed)	Negative
		FVB/N-Tg(vHRas)4Lep*	(feed)	Negative
2	Acesulfame	B6.129-Trp53tm1Brd	0%, 0.3%, 1%, or 3% (feed)	Negative
		FVB/N-Tg(vHRas)4Lep*	0%, 0.3%, 1%, or 3% (feed)	Negative
3	TMPTA	FVB/N-Tg(vHRas)4Lep*	0, 12.5, 25, 50, 100, or 200 (topical, acetone)	Positive*
4	PETA	FVB/N-Tg(vHRas)4Lep*	0, 12.5, 25, 50, 100, or 200 (topical, acetone)	Positive
5	BDCM	B6.129-Trp53tm1Brd	0, 175, 350, or 700 mg/L M (DW or gavage)	Negative
		FVB/N-Tg(vHRas)4Lep*	0, 175, 350, or 700 mg/L M (DW or gavage)	Negative
6	Na Bromate	B6.129-Trp53tm1Brd	0, 80, 400, or 800 mg/L (DW)	Negative
		FVB/N-Tg(vHRas)4Lep*	0, 80, 400, or 800 mg/L (DW)	Negative
11	DCA	B6.129-Trp53tm1Brd	0, 500, 1000, or 2000 mg/L (DW)	Negative
		FVB/N-Tg(vHRas)4Lep*	0, 31, 125, or 500 mg/kg (topical, acetone)	Positive
		FVB/N-Tg(vHRas)4Lep*	0, 500, 1000, or 2000 mg/L (DW)	Positive
12	Phenolphthalein	B6.129-Cdkn2atm1Rdp	0, 200, 375, 750, 3,000, or 12,000 ppm	Negative
13	Glycidol	B6.129-Cdkn2atm1Rdp	0, 25, 50, 100, or 200 mg (gavage, DI water)	Positive

Trimethylolpropane Triacrylate

Pentaerythritol Triacrylate

Bromodichloromethane

Dichloroacetic acid

*BSC did not consider this model a carcinogenicity study



Key Issues

- Selection of inbred strains
- Epistasis
- Genetical genomics, proteomics, and metabolomics
- Meiotic mapping of resistant and susceptible strains
F1 intercross (F2) for QTL analysis



Hypotheses

- 1) Tumor spectrum, prevalence, and latency,
- 2) Transcript, proteomic, or metabolomic expression profiles, (corroborated by copy number variation)

Will segregate according to the haplotype of p53 haploinsufficient F1 hybrid isogenic lines selected on the basis of genetic variation in DSB repair genes associated with genomic instability

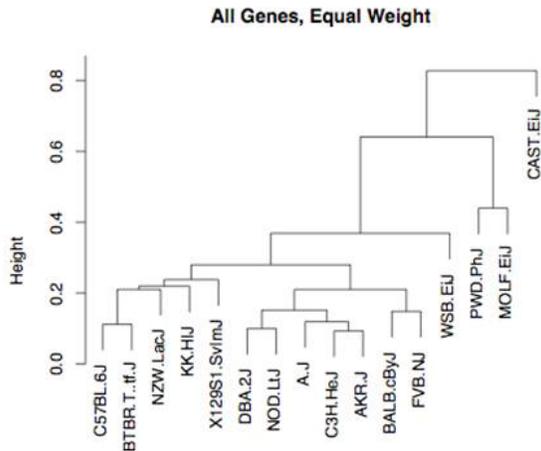


Aims

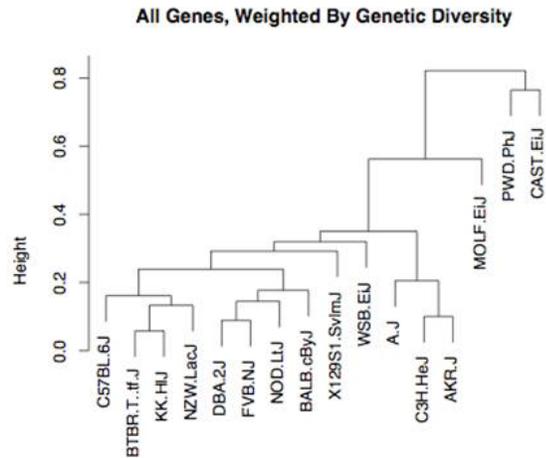
- **Aim 1.** Outcross A/J, BALB/C, BTBR.T/J, C3H/HeJ, DBA2/J, or 129S1.SvImJ X B6.129-*Trp53*^{tm1Brd}
- **Aim 2.** Expose each F1 progeny to 0, 3, or 6 Gy ($\pm 10\%$ total dose) ionizing radiation to induce DNA strand breaks, DNA damage, and tumorigenesis
- **Aim 3.** Perform gross necropsy on all F1 hybrid and collect blood (at exsanguination) and samples from target tissues and gross malignant tissue analysis
- **Aim 4.** Determine the prevalence of tumor phenotypes, tumor latency, genome wide allele specific loss, genome-wide CNV, transcript expression profiles, and serum metabolomic differences between tumors and histological normal tissue (germline control)
- **Aim 5.** Identify strain specific changes in CNV, allele specific loss, transcript expression profiles and the statistical association with tumor phenotype and prevalence
- **Aim 6.** Perform bioinformatic and comparative genomic analysis of common or strain specific genes associated with ionizing radiation induced tumor phenotypes and identify any orthologous human genes

All Genes, Equal Weight

All Genes, Weight By Diversity



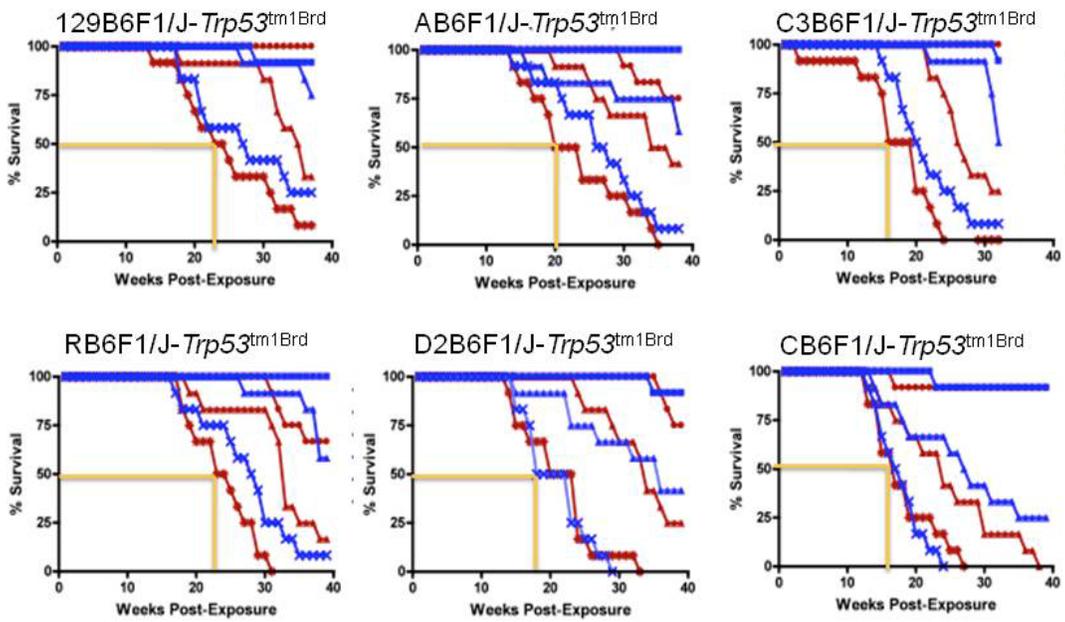
1 - avgMat
Hap Size = 3



1 - weightedMat
Hap Size = 3

Strain selection based upon genetic diversity of NHEJ DSB repair pathway:
 Female C57BL/6J, BTBR.T/J, DBA2/J, BALB/C, 129S1.SvlmJ, A/J, or C3H/HeJ x male
 B6.129-*Trp53*^{tm1Brd}(+/-).

Survival Analysis



Strain selection based upon genetic diversity of the DSB repair pathway:
 Female C57BL/6J, BTBR.Tf/J, DBA2/J, BALB/c, 129S1.SvlmJ, A/J, or C3H/HeJ x male
 B6.129-*Trp53*(+/-).
 (males – 0-●, 3-□, or 6-★ and females - 0-●, 3-□, or 6-★ - gamma irradiation)



Significance and Expected Outcomes

- C3B6F1 p53 haploinsufficient mice have shown a non-random allele specific loss associated with *Melm3*, *Trp53*, and *Rad51c* genes
- Survival and tumor phenotypes observed by histopathology are significantly different between the p53 deficient F1 hybrid mice
- Development of a short-term (39 wk) cancer bioassay in genetically diverse F1 mice could reduce the potential false negative results and improve confidence
- F1 intercross to produce p53 haploinsufficient F2 progeny for a short term IR tumorigenesis study might be used for meiotic mapping and identification of QTLs and human orthologs



Current and Future Plans

- Determine the potential for short-term cancer bioassays to test hypotheses based outcomes with NTP-nominated chemicals
- Results may indicate that p53 deficient F1 or other pathway deficient strains can be used for GAM models
- Test F1 hybrids in short-term cancer bioassay with model genotoxic and non-genotoxic NTP chemicals
- Meiotic mapping association studies to identify QTLs (candidate genes) and human orthologs
- Use F1 wildtype and F1 TSG deficient strains for short-term (28 or 90 day) exposures to identify intermediate phenotypes for predicting carcinogenic potential (see Project 8).



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Questions/Comments

